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Studies Using Synthetic Diets for Dairy Calves

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Young calves have been fed a synthetic type milk diet to obtain information on their dietary needs for vitamin B_{12}, pyridoxine and alpha tocopherol. The semi-synthetic milk diets were fed to calves maintained in metal metabolism cages starting on the third day of life. They were fed milks at the rate of 10 to 12 lb. per 100 lb. live weight. Synthetic type milk #1 was made according to the method described by Clark (1) and milk #2 was made according to the method described by Weise and associates (2). Both contained 3.69 lb. cerelose (corn sugar), 1.7 lb. lactose, 3.89 lb. lard and 4.35 lb. casein per 100 lb. liquid milk. The casein in milk #2 had been extracted with hot ethanol. Minerals were added to furnish the amounts of the elements listed below (as percent or ppm of the liquid milk).

	Milk #1	Milk #2	
Calcium	0.151	0.161	%
Phosphorus	0.097	0.081	"
Sodium	0.057	0.054	"
Potassium	0.156	0.165	"
Iron	0.0026	0.0030	"
Magnesium	0.006-0.022-0.036	0.001-0.011-0.036	"
Chlorine	0.097	0.099	"
Sulfur	0.048	0.005	"
Manganese	5.6	5.4	ppm.
Copper	1.9	2.6	"
Zinc	0.6	0.6	"
Fluorine	0.6	0.6	"
Iodine	3.4	3.4	"
Cobalt	0.0 & 0.3	0.3	"

The amounts of the vitamins given the calves are indicated below.

Thiamine	2.95	mg./day/100 lb. body weight
Riboflavin	5.91	" " " " " "
Pyridoxine	0 or 2.95	" " " " " "
Ca-Pantothenate	18.2	" " " " " "
Inositol	236.2	" " " " " "
Choline	1,181.0	" " " " " "

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P.A.B.A.	11.8	mg./day/100 lb. body weight
Folic acid	0.7	" " " " " "
Biotin	0.09	" " " " " "
Niacin	11.8	" " " " " "
Ascorbic Acid	59.0	" " " " " "
2-Methyl,1-4aphthquinone	1.2	" " " " " "
Alpha Tocopherol 4.5 or 10 or 20 or 70		" " " " " "
Vitamin B_{12} 0 or 18 or 57		ug./day/100 lb. body weight
Vitamin A	25,000 I.U.	" " " " " "
Vitamin D	430 I.U.	" " " " " "

Milk #2 containing no vitamin B_{12} by rat assay was fed to 15 calves to study the requirement of the calf for this vitamin. However, some difficulty with vitamin E deficiency was encountered because it was not realized that the addition of tocopherol to the milk to furnish 4.5 mg./cwt. was an insufficient amount of tocopherol. Consequently the first 10 calves on this diet died of tocopherol deficiency at ages ranging from 87 to 157 days. Five other calves have received alpha tocopherol at 70 mg./day/cwt. No cobalt was added to the B_{12} deficient milk. One calf died of hyperthermia of undetermined cause at 172 days of age, and one calf is still on experiment at 120 days of age. Three calves were slaughtered at 239 to 326 days of age.

A slow rate of growth has been the most noticeable symptom shown by calves on this ration. It became very pronounced as the calves grew older because of anorexia. The monthly gains in body weight of these calves as compared to normal weight gains (3) are shown in the upper portion of table 1. Hemoglobin values have always been within the normal range.

Fresh fecal passages were collected, autoclaved, dried and ground for assay of vitamin B_{12} by the rat method. It contained from 0.2 to 0.5 ug. B_{12}/gram dry matter at 30 to 146 days of age. The concentration did not increase with length of time on the diet. Total collections were not obtained but it was estimated that the daily excretion was about 20 ug./day. The liver of one calf slaughtered at 239 days of age contained 2.3 ug. vitamin B_{12} per gram dry liver tissue. Two calves on this diet showed increased rates of growth when vitamin B_{12} was added to the milk to furnish 40 ug. B_{12}/day/cwt.

Fourteen calves were fed milk #2 with no added vitamin B_6 (pyridoxine) to study the omission of this vitamin from the diet. Nine calves fed the milk without added pyridoxine have died at 46 to 223 days of age, and three lived for an extended period of time. One was slaughtered at 357 days of age, another at 595 and the other was removed from the diet at 560 days of age. These three calves always gained at a faster rate than the other nine calves and at 9 and 10 months of age they averaged 66% of normal body weight. At these ages they appeared normal except for size. The two surviving calves gained very little after one year of age.

The calves on this diet had a slow rate of growth. Their comparative gains each month are shown in the center portion of table 1. Five of these calves had uremia at time of death. They were all in an emaciated condition and reduced amounts of body fat were noted at autopsy. Only one calf had convulsive seisures; these occurred when it was three months of age. After this time it was normal until slaughtered at 357 days of age.

Two calves were fed milk #2 and injected with desoxypyridoxine at 10 mg. per day from 3 to 20 days of age. After 20 days of age they received about 20 mg./day. They died at 11 and 59 days of age. After injection of a dose larger than 20 mg. the surviving calf would become listless and limp. Omitting the injection of the desoxypyridoxine the next day usually alleviated this condition. Otherwise the calves appeared essentially normal except for intermittent diarrhea until a day before death when they became very listless and limp. They were in a semi-comatose condition at death. It is not known whether death was due to toxicity of the drug per se or due to its anti-vitamin effect.

One calf fed only whole milk and a similar amount of desoxypyridoxine had diarrhea during the period the anti-vitamin was given. The diarrhea stopped when the injection was omitted for two days and ceased entirely when injection of the desoxypyridoxine was discontinued at 60 days of age.

A few determinations on the total pyridoxine content of tissues was made by the method described by Parrish et al. (4). One calf receiving the unsupplemented milk had 3.1 ug. vitamin B_6 activity per gram of dry liver tissue. Two calves, one receiving B_6 supplemented milk and the other a normal diet, had 32 and 26 ug. B_6 activity per gram of dry liver tissue, respectively. The concentration of B_6 activity in the blood of three calves receiving the unsupplemented milk was 5 to 9 ug./100 ml. plasma and the concentration in the plasma of supplemented calves or calves on normal diets was 9 to 46 ug./100 ml. One calf that remained on the unsupplemented diet past 500 days of age had 15 and 16 ug. B_6 activity/100 ml. plasma at 470 and 500 days of age respectively. Apparently this calf was obtaining some of the vitamin from synthesis, probably within the gastro-intestinal tract. The urinary excretion of this vitamin by the calves on the unsupplemented milk was 0.3 to 1.9 mg. B_6 activity per day compared to 1.3 to 2.5 for the supplemented calves or calves on normal diets. One of the calves receiving the unsupplemented milk plus desoxypyridoxine excreted only 0.1 to 0.2 mg. B_6 activity per day. It is evident that the B_6 activity in the liver, blood plasma and urine of these calves was lower than that of calves receiving the same milk supplemented with pyridoxine. The microbiological assay indicated there was a small amount of pyridoxine active material in the milk. It was considerably reduced when ethanol extracted casein was used in place of regular casein.

From the limited data on the B_{12} and B_6 content on the tissues of these calves it is evident that a complete deficiency of the vitamins was not produced with this milk diet. Male calves were used in these studies and on these diets the calves would often drink their own urine as it was voided which would recycle small quantities of the vitamin under study.

Milk #1 was used in studies that involves magnesium and tocopherol but only the latter results will be discussed in this report.

The calves on the semi-synthetic milk diet that had alpha tocopherol added to the milk to furnish 4.5 mg. per cwt. died at 87 to 160 days of age. The first symptom observed was decreased rate of drinking which was apparently caused by difficulty in swallowing. Soon after this the calves had difficulty in getting up, and when they were helped up they could not walk. Often they had involuntary muscle twitching. The respiration rate and muscle twitching or spasms were exaggerated after the slightest exercise. Most calves would consume milk slowly while lying down. Electrocardiographs of two calves taken before the paralysis was severe were essentially normal. It was impossible to obtain electrocardiographs when the calves had muscular spasms.

Four calves received alpha tocopherol at the level of 10 mg./day/cwt. They remained essentially normal until shortly before their death which occurred at 155 to 240 cays of age. At this time the average body weight of these calves was 82% of the standard (3). These calves had symptoms of paralysis and local or generalized spasms similar to the calves on the lower intake.

After a few days of paralysis one calf collapsed and could not get up. It was injected with 200 mg. of alpha tocopherol phosphate daily for the next two weeks. In four days it was able to rise and stand for 30 seconds, in seven days it could take a few steps and in 11 days it was nearly normal in its activity.

Two of the calves had very white muscles, one had gross calcification in the muscles and the other had increased calcium content in the white areas of its heart muscle. All calves had hyperthermia at death. Plasma tocopherol was 0.050 mg./100 ml. in one calf.

The tocopherol level in the synthetic milk was then increased to 20 ug./day/cwt. Plasma tocopherols were below normal on this level of intake. It decreased below 0.100 mg./100 ml. by the time the calves were three months of age. It appeared that the addition of this amount of tocopherol to the milk provided insufficient amounts of tocopherol to the calf. It is likely that some of the tocopherol added to the milk as it was made was destroyed and it is possible that the added ferric salts were active in this destruction. Calves given additional tocopherol (50 mg./day) by capsule have maintained adequate plasma tocopherol levels (5).

This study has indicated the difficulty of producing a complete deficiency of vitamins B_6 and B_{12} (and other 'B' vitamins) in young calves on a semi-synthetic milk type diet. A dietary need for these vitamins was indicated by the reduced growth rate and tissue storage but this technique did not produce a complete deficiency in the animals. Symptoms of a alpha tocopherol deficiency are described as it occurs in young calves.

Table 1.- Growth[1/] of Calves Fed Semi-Synthetic Milk Diet Low in Vitamins B_6 and B_{12}

1	2	3	4	5	6	7	8	9	10
Low B_{12}									
35 (17)	42 (15)	46 (13)	33 (7)	24 (4)	20 (3)	32 (3)	32 (3)	75 (1)	22 (1)
Low B_6									
41 (12)	48 (9)	52 (8)	45 (6)	45 (4)	42 (4)	45 (4)	57 (3)	49 (3)	64 (3)
Adequate in above vitamins									
91 (5)	81 (6)	82 (6)	82 (6)	78 (6)	70 (3)	85 (3)	-	-	-

1/ Body weight gains as percent of normal for the respective breed and sex (3) for each month of age. Figures in parenthesis indicate the number of calves.

(1) W. M. Clark: J. Dairy Sci. 27:195. 1927.
(2) A. C. Weise et al.: J. Dairy Sci. 30:87. 1947.
(3) C. A. Mathews and M. H. Forhman: U.S.D.A. Tech. Bul. 1098 and 1099. 1954.
(4) W. P. Parrish et al.: J.A.O.A.C. 38:506. 1955.
(5) J. W. Thomas and M. Okamoto: U.S.D.A. A.R.S. 52-12. 1955.